BOTANICAL GAZETTE

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DIFFERENTIATION OF SEX IN THALLUS GAMETOPHYTE AND SPOROPHYTE.[1]

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(WITH PLATE VI AND THREE FIGURES)

IN a recent article (**5**) the writer has given a somewhat detailed account of zygospore germinations in certain species of the Mucorineae. The purpose of the present paper is to point out the bearing which the investigations already made in this group may have upon the questions of sexuality in other forms. Some of the problems for research which the facts observed in the mucors would suggest will be indicated, and it is hoped that in forms in which an alternation of generations occurs the distinction between differentiation of sex in the gametophyte and that in the sporophyte will be more clearly drawn than has been done previously. The various grades of differentiation in the gametes themselves or in the gametophyte and sporophyte will not be discussed. The subject for consideration rather will be the sexual condition in the plant as a whole.

According to the sexual character of their thalli, the species of the Mucorineae have been divided (**2–6**) into two main groups, homothallic and heterothallic—designations which correspond in the main to the terms hermaphroditic and dioecious respectively. In a homothallic species the thalli are all sexually equivalent, while in a heterothallic species the thalli are of two different kinds, which have been provisionally designated by the symbols (+) and (−). The sexual character of the (+) and (−) mycelia remains constant when

[1] This paper was written while working under a grant as research assistant of the Carnegie Institution, to whom the writer wishes to express his indebtedness for the opportunities for research afforded him.

they are grown separately in pure cultures. Thus the opposite strains of *Phycomyces* and *Mucor Mucedo* have been cultivated by means of sporangiospores to respectively 107 and 106 non-sexual generations without apparent change in their sexual behavior. This differentiation into (+) and (−) mycelia, which are capable of retaining their respective characters apparently for an indefinite number of vegetative generations, renders the heterothallic mucors as striking an example of dioecism as is to be found in the plant kingdom.

In those heterothallic species investigated in which a difference in vegetative growth is apparent, the (+) strain is the more luxuriant. In higher forms when a difference in size exists between the two sexes, the female is usually the larger. In such heterothallic forms the zygote develops entirely from the female thallus, and it would not seem unnatural that the thallus which supplies nourishment for the formation of the reproductive bodies should have a greater development than the thallus which produces only the comparatively small male gametes. The zygote of the heterothallic mucors, on the other hand, is formed by the union of morphologically equal gametes cut off from similar branches of the sexually opposite thalli. The zygospore is suspended midway between the (+) and (−) thalli which take equal share in supplying the nutriment for its development. The difference which sometimes exists in vegetative luxuriance between the two strains is independent therefore of the demands of the reproductive bodies, and is to be connected in some way with the primary sexual differentiation into the two opposite strains.

There are no heterothallic species as yet known in which a constant difference between the size of the two gametes has been observed. Two genera from the homothallic group are heterogamic, and in these forms the smaller gamete may be assumed to be male and the larger female. If it were found that a (+) test strain would show a reaction with the male, while the (−) strain showed a reaction with the female branch, one would have evidence for considering the (+) strain female and the (−) strain male. Unfortunately, attempts to hybridize test (+) and (−) heterothallic strains with these heterogamic forms have been as yet entirely unsuccessful. It is to be hoped that other heterogamic forms may be discovered which will lend themselves more readily to experiments in hybridization. That

as yet it has not been possible to substitute the terms male and female for (+) and (−), or *vice versa*, does not in the least detract from the conclusion, however, that the differentiation is a sexual one.

Forms characterized by gametes equal in size have been commonly classified as isogamous. The term, it need hardly be pointed out, can have only a morphological application among the mucors. Sexually the two gametes which unite have diametrically opposite characters. The mutual indifference of two mycelia of the same sex, and the active sexual reaction between mycelia of opposite sex which leads to the formation of zygospores when the mycelia are of the same species, and to the formation of imperfect hybrids when they are of two different species, indicate that the isogamy is by no means physiological. The classical researches of BERTHOLD (1) have shown that among the morphologically equivalent motile gametes of certain species of Ectocarpus there is a physiological differentiation into gametes which are attractive and those which are attracted, and a similar condition is met with among the Conjugatae. In the mucors the sexes seem to be equally attractive. If in other zygophytic forms the gametes are ever physiologically equivalent, their union can scarcely be considered a sexual process in the usual acceptation of the term.

The physiological differences which exist between the sexually opposite thalli of heterothallic mucors reaches morphological expression in those instances in which the (+) in comparison with the (−) strain is characterized by a greater vegetative luxuriance. Although the heterothallic forms are morphologically all isogamous, the sexual differentiation which they exhibit into two distinct races cannot be considered a lower grade of sexuality than the differentiation shown in the morphologically unequal gametes of the heterogamic species. Heterogamic forms are found only in the homothallic group. It would seem most reasonable to suppose that the isogamous homothallic forms were the more primitive, and had given rise on the one hand to heterogamic forms by a differentiation of the individual gametes, and on the other hand to heterothallic forms by a differentiation of the individual thalli. The partial transformation of the heterothallic species Phycomyces into a homothallic form which has been accomplished might, however, suggest the possibility of a deri-

vation of the homothallic forms from the heterothallic group. There are seven species known to be homothallic, among which three are heterogamic, while sixteen are known to be heterothallic. In all probability the large majority of the species which produce zygospores are heterothallic, yet the sexual character in but a small proportion of the mucors has been definitely determined, and it is unknown whether in this group species may not exist in which sexuality is entirely lacking. The writer has as yet no theories to offer as to the origin of sexuality in the group.

The fact that zygospores when germinating in a proper nutrient medium may give rise directly to a mycelium has led botanists to discard the idea of an alternation of generations comparable to that in higher plants, which was formerly seen in the succession from mycelia bearing sexually formed zygospores to germ tubes producing non-sexual sporangiospores which complete the cycle by the formation again of sexual mycelia. The cytological history of the formation and germination of the zygospores is at present too little known, and the writer would not care to be responsible for advocating as yet a too close homology between the conditions seen in the mucors and in the mosses for example, although the branching out of the germ tube under special conditions to form a mycelium might be considered of no great significance, since paralleled by the capacity of the moss sporophyte to give rise directly to a protonema. The gross analogy, however, between the germination of the zygote in mucors and that in the mosses is much more obvious than between the conditions in the mosses and those in the flowering plants or in animals (**9**), and is sufficiently close to justify one in concluding the mucors in a general comparison of the varying grades of sexual differentiation in the plant kingdom. In the accompanying diagrams and in the ensuing discussion, therefore, the same terminology will be applied to the mycelium and to the germ tube that has been found advisable for the gametophyte and sporophyte of forms in which it is at present orthodox to speak of an alternation of generations.

The terms dioecious, monoecious, and hermaphroditic have been used to designate varying grades of sexual differentiation, and have been applied to both gametophyte and sporophyte. Dioecism among

the bryophytes has been understood to signify the existence of two kinds of gametophytes, male and female, and the condition in the sporophyte has been disregarded; while among the flowering plants the usage is changed and dioecism has had reference solely to the sporophyte. An inspection of the accompanying diagrams will show that a plant which is monoecious as regards its sporophyte may be either monoecious or dioecious as regards its gametophyte; and on the other hand a plant dioecious in its gametophyte stage may be either monoecious or dioecious in its sporophyte stage. The first case is illustrated by the ferns, which are all dioecious in the sporophyte though having both conditions in the gametophyte; and the second case is illustrated by the flowering plants, whose sporophytes are either monoecious or dioecious, but whose gametophytes are always dioecious. In flowering plants and in ferns, one of the two generations is characterized by only a single sexual condition, and attention has accordingly been directed to the other generation in which both sexual conditions are present. That this inaccuracy in the terminology has been allowed to stand so long unchallenged is probably due to the tacit assumption that the condition in the ferns is typical for all the archegoniates. Up to the present time, however, the sexual condition in the sporophyte of forms below the ferns has never, so far as the writer is aware, been a subject of investigation or even of discussion.

The terms hermaphroditic, monoecious, and dioecious have established themselves in use, and have their place as technical designations in systematic botany of the flowering plants. As applied to the cryptogams, they have always been unsatisfactory, since the terms hermaphroditic and monoecious are used in descriptive botany to indicate whether the male and female sporophylls are produced in bisexual or unisexual flowers. In the cryptogams the terms lose their distinction with the passing out of use of the word flower. The greater or less local separation of the sexual organs or of the male and female sporophylls on a single individual is of little significance in comparison with the separation of the sexes on two entirely distinct individuals. Whether in Achlya, for example, the antheridia arise from the stalk which bears the oogonium as in *A. racemosa*, or are produced from separate special branches as in *A. prolifera*, is a

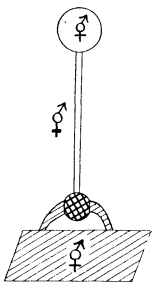
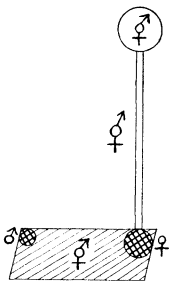
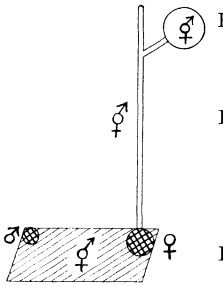
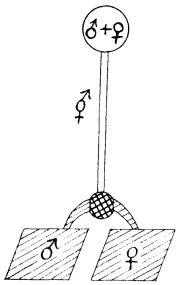
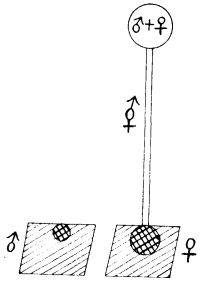
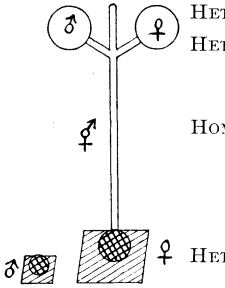
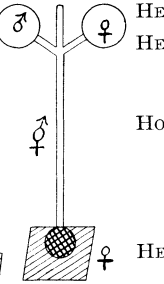
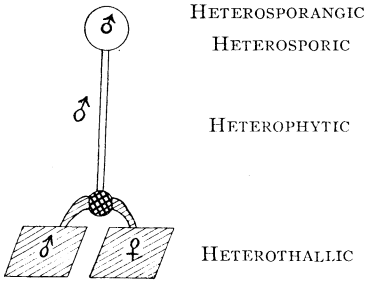
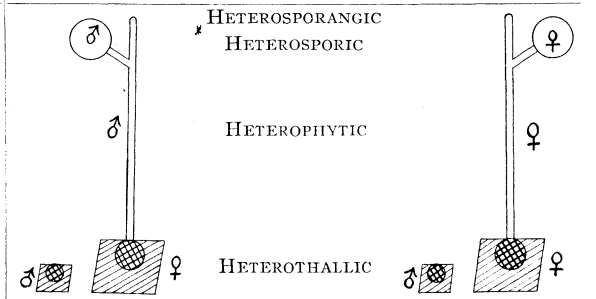
detail of somewhat minor importance. The sexual differentiation on a single mycelium in the latter species may be perhaps a forerunner of heterothallism, yet in each species the thallus as a whole is bisexual.

Rather than attempt to restrict the terms monoecious and dioecious to either the gametophytic or sporophytic stage, it has seemed best for the purposes of general discussion in the present article to avoid the ambiguity of the expressions now in use by applying a separate set of terms to designate the sexual condition in the gametophyte and sporophyte respectively. Whether or not the precision thereby gained will compensate for the disadvantages of adding new words to an already overburdened vocabulary of technical expressions must rest with botanists whose interest in the subjects of sexuality embraces all the groups of the plant kingdom.

Homothallic and heterothallic are terms already explained, which the writer has used to designate the species of the mucors characterized respectively by thalli sexually all alike, or by thalli sexually of two different kinds. Homothallic and heterothallic forms, therefore, have bisexual and unisexual thalli respectively, and the terms accordingly would correspond to the expressions monoecious and dioecious. Without changing the etymological significance, the meaning of the words homothallic and heterothallic may be appropriately extended to include a description of the degree of sexual differentiation in the prothallus or gametophyte of the archegoniates and spermatophytes, as well as in the thallus of the thallophytes.

Homophytic and *heterophytic* are offered as equivalents in the sporophyte of the terms monoecious and dioecious. Although the "plant" in the common acceptation of the word is the sporophyte in the higher forms, the condition is reversed in the bryophytes. The words homophytic and heterophytic, therefore, as designations for the sporophyte are etymologically not above reproach, but will suffice in lieu of a more cumbersome combination. The terminology suggested has reference to the sexual differentiation as such. The accompanying morphological differences are to be considered as secondary sexual characters and are not included in the classification.

It will now be possible to examine the sexual condition in the

MUCORINEAE	BRYOPHYTES	PTERIDOPHYTES	PHANEROGAMS
SPORODINIA	PHYSCOMITRIUM	POLYPODIUM	
 HOMOSPORANGIC HOMOSPORIC HOMOPHYTIC HOMOTHALLIC	 HOMOSPORANGIC HOMOSPORIC HOMOPHYTIC HOMOTHALLIC	 HOMOSPORANGIC HOMOPHYTIC HOMOTHALLIC	
PHYCOMYCES	MARCHANTIA	SELAGINELLA	LILIUM
 HOMOSPORANGIC HETEROSPORIC HOMOPHYTIC HETEROTHALLIC	 HOMOSPORANGIC HETEROSPORIC HOMOPHYTIC HETEROTHALLIC	 HETEROSPORANGIC HETEROSPORIC HOMOPHYTIC HETEROTHALLIC	 HETEROSPORANGIC HETEROSPORIC HOMOPHYTIC HETEROTHALLIC
MUCOR MUCEDO			POPULUS
 HETEROSPORANGIC HETEROSPORIC HETEROPHYTIC HETEROTHALLIC			 HETEROSPORANGIC HETEROSPORIC HETEROPHYTIC HETEROTHALLIC

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groups represented in the accompanying diagrams. In all the figures the gametophyte has been shaded with parallel lines, the antheridia and zygotes with cross-hatching; while the sporophyte and the sporangia have been left unshaded. The drawings are entirely diagrammatic, and no attempt has been made, therefore, to preserve the relative proportions of the parts figured. As has been already explained, the Mucorineae have been included in this scheme for the purpose of comparison, and the germ tube has been thus homologized with the sporophyte. The mucors then as represented in the first column in the diagram are the only group outlined in which all the three main types of sexual differentiation are as yet known.

In *Sporodinia grandis*, which may be taken as representative of the homothallic group, the mycelium (gametophyte), the germ tube (sporophyte), and the germ sporangia are all alike bisexual. The two opposed gametes, and perhaps the branches from which they are cut off, may not unreasonably be considered unisexual and of opposite sex. It has not been found possible as yet, however, to confirm this assumption experimentally. In the terminology adopted the species is to be considered homothallic, homophytic, homosporic, and homosporangic. The same condition is found in the "monoecious" mosses represented by *Physcomitrium pyriforme*, and in the homosporous ferns represented by Polypodium. The sporangium of the latter is represented as a side branch, since in the ferns, as also in the flowering plants, the sporangia are not simple terminations of unbranched sporophytes of limited growth, as in the bryophytes, but are borne on the sporophylls of a sporophyte more or less highly developed.

If the sexual character of the thallus be preserved, the spores and the sporophyte producing them must be also bisexual. There can be only one type therefore of homothallic forms. Of heterothallic forms, on the contrary, two types are possible—namely, those with bisexual sporophytes, *i. e.*, homophytic, and those with unisexual sporophytes, *i. e.*, heterophytic. These two types are represented by *Phycomyces nitens* and *Mucor Mucedo* respectively.

In the heterothallic species Phycomyces it will be convenient for the purposes of the present paper to neglect those instances in which the germination follows the *Mucor Mucedo* type, as well as the

occasional formation of homothallic spores in the germ sporangia, and to consider as typical the condition shown in the diagram. For a more detailed account of the zygospore germination in Phycomyces, as well as for the characters of the homothallic form into which this heterothallic species has been transformed, one must refer to the paper on zygospore germinations already cited. In the type, perhaps somewhat arbitrarily selected for discussion, the germinations are mixed—both male and female spores being produced in a single germ sporangium. The mycelia in this species are unisexual, the zygospores and germ tubes are bisexual, and the spores in the germ sporangia are unisexual. If the germ tube be forced to form a mycelium without the intervention of sporangiospores, a bisexual, *i. e.*, homothallic, mycelium results, which may produce typical homothallic zygospores. Phycomyces as discussed, therefore, is heterothallic, homophytic, heterosporic, and homosporangic.

In the bryophytes, *Marchantia polymorpha* is the only form which has been investigated in regard to the sexual condition of its sporophyte (cf. p. 170). Its gametophyte shows a differentiation into male and female thalli, and the germination of the zygote produces a sporophyte which bears a sporangium containing both male and female spores. Marchantia, therefore, like Phycomyces is heterothallic, homophytic, heterosporic, and homosporangic.

Selaginella, as a representative of the heterosporous ferns, follows in the main the Phycomyces type. It differs from Phycomyces and Marchantia, however, in that it is heterosporangic—the male and female spores being separated in microsporangia and macrosporangia. The spores themselves, moreover, are morphologically of two kinds, the female or macrospores being conspicuously larger than the male or microspores. This morphological differentiation of the spores and sporangia is known only in the heterosporous ferns and in the flowering plants, and is accompanied by a reduction in the size of the gametophyte. Among the homosporous ferns, prothalli are often found with only archegonia or antheridia, and investigators have been able to suppress the formation of one or the other in certain species where archegonia and antheridia occur normally side by side on the same prothallus. The writer is aware, however, of no form

among the homosporous ferns which investigators have shown to be strictly heterothallic.

In the monoecious and hermaphroditic phanerogams, illustrated by Lilium, the condition is essentially the same as in Selaginella, with a differentiation into macrospores and microspores, and like the latter species the type may be described as heterothallic, homophytic, heterosporic, and heterosporangic.

The homophytic division of the heterothallic group illustrated by Phycomyces is the only one of the three types that has representatives in all the orders outlined.

Mucor Mucedo represents the heterophytic division of the heterothallic group. In contrast to Phycomyces, the zygospores of this heterothallic species furnish pure germinations, but the spores are unisexual; and while the germ tube and the sporangiospores produced from one zygospore are male, those produced from another may be female. There are, therefore, two different kinds of germ tubes, of sporangiospores, and of sporangia, as well as two different kinds of mycelia. These elements in this species show no more recognizable morphological differences than its mycelia, although the sexual differentiation seems to be as marked as in forms in which such a morphological differentiation exists throughout the whole plant. *Mucor Mucedo* is heterothallic, heterophytic, heterosporic, and heterosporangic.

Since Marchantia is the only heterothallic form among the bryophytes the sexual character of whose sporophyte has been investigated, it is as yet unknown whether any forms of the mosses and liverworts exist corresponding to the *Mucor Mucedo* type.

No heterophytic forms are known at present among the heterothallic pteridophytes, and it will be impossible to say whether they ever existed in geologic times. The non-appearance of one reproductive form on a given sporophyte cannot be taken at once as proof that the species is heterophytic. It not infrequently happens, for example, that one finds only microsporangia on a single individual of Selaginella. Such instances may be compared to the suppression of the organs of one sex on the prothalli of homothallic ferns, and may equally be explained by assuming that the conditions necessary for the formation of the two reproductive bodies do not always coincide.

The "dioecious" phanerogams, represented by the heterophytic form Populus, follow closely the *Mucor Mucedo* type. They differ from *Mucor Mucedo* in that the sexual differentiation has reached a morphological expression, and the sex of the thalli, spores, and sporangia is at once distinguishable. In general the male and female sporophytes are alike in appearance, but in the sporophytes of some forms the sexes are easily distinguished. Perhaps the best known example among the common trees is the Lombardy poplar (*Populus pyramidalis*), which in male specimens has been widely cultivated for the sake of its pyramidal form. The female trees have a spreading habit of growth and are seldom to be found in cultivation.

In the diagram three squares are left blank. In the flowering plants heterothallism has become fixed and no forms of the Sporodinia type exist. There is no reason apparent why heterophytic forms should not occur among the heterothallic pteridophytes. The fact remains that all the existing heterothallic species are homophytic. The blank squares in the phanerogams and pteridophytes must therefore remain unfilled. Little is known about the sexual differentiation in the bryophytes, and it must rest with future research, therefore, to determine whether or not they possess heterophytic representatives in the heterothallic group.

In light of the conditions found in the Mucorineae, the heterothallic bryophytes, as already pointed out by the writer (*l. c.*, p. 25), offer a most interesting field for investigation. Accordingly attention was directed to the heterothallic form *Marchantia polymorpha*, which, according to the unpublished observations of NOLL as reported by SCHULTZE (**17**), retains the unisexual character of the individual thalli when propagated vegetatively by gemmae.

During the last November, Marchantia was found in fruit and sowings were made from individual sporangia, and the young plants resulting from their germination were isolated and transplanted in such a manner that at fructification it would be possible to determine the sex of the individual spores from which they were derived. While the present paper was largely in manuscript, the writer learned of unpublished observations made by NOLL on this same species. Professor NOLL, to whom the writer is greatly indebted for the information communicated, has cultivated Marchantia by means of gemmae

for over thirty generations of both male and female strains, without having been able to change the sexual character of the thalli by subjecting them to varying conditions of growth. The form is therefore strictly heterothallic. Moreover, in a single instance a sporangium was made to discharge its spores on a pot of earth, and male and female fructifications were obtained from the mixed growth of thalli resulting from their germination. Marchantia is therefore homophytic, and it now becomes possible to fill out in the diagram one of the two squares which in the bryophytes had been left blank pending the fructification of the young thalli which the writer had obtained from isolated spore germinations.[2]

In Phycomyces, with which Marchantia is to be compared, there seems to be no definite relation between the number of male to female spores formed in a germ sporangium, and it may even happen that all the spores are of the same sex. Moreover, it is not infrequently the case that in a small per cent. of the spores in a germ sporangium the segregation into male and female has not been completed. These bisexual spores produce homothallic mycelia. Cultures from individual spores will be necessary to determine for Marchantia the proportion of male to female spores in a single sporangium, and to ascertain if, in addition to the normally unisexual spores, bisexual spores are ever formed, as is the case in Phycomyces.

The bisexual germ tube of Phycomyces may be cut and forced to branch out to a homothallic mycelium. The observations of NOLL and of the writer have shown Marchantia to be homophytic. Its sporophyte as a whole, therefore, must be bisexual, and every cell formed before the determination of the sex of the spores, if brought to develop into a new plant, should theoretically produce homothallic individuals. PRINGSHEIM (16), STAHL (18), and CORRENS (10), among others, have obtained protonemata from the sporophytes of mosses. No one, however, seems to have succeeded in obtaining regeneration from the sporophyte of liverworts. The writer has experimented with mature sporophytes of Fegatella and with sporophytes of Marchantia of various ages, but has been unable to secure any growth from them.

[2] While the present paper is in press, 12 thalli have so far produced fructifications out of a total of 113 which were obtained from as many spores from a single sporangium. Of these nine are male and three are female.

In the investigation of the typical germinations of Phycomyces, it has been shown that the determination of sex does not occur in the zygote, but that an interval in the form of a germ tube is interpolated between the zygote and the germ sporangium where the segregation of sex finally occurs. The essential difference between Phycomyces and Marchantia lies in the fact that in the former the interval is a single-celled multinucleate structure arising from a multinucleate zygospore, while in the latter the interval is made up of many uninucleate cells arising from a uninucleate oospore. In Marchantia the segregation of sex undoubtedly takes place at some point in the maturation of the sporangium. If the archesporium and the spore mother cells prove capable of germinating, and it be possible in the thalli which result to recognize the presence of both sexes when the plants are homothallic, one may be in a position to determine the exact point where the segregation of sex occurs and to discover what relation if any the segregation may have to the reduction division or to other nuclear phenomena.

The predominance of organs of a single sex on the prothallus of the ferns due to conditions of growth and the similar phenomenon in the sporophyte of Selaginella may lead to the non-appearance of the other sex. Such a suppression of sex, however, is not to be confused with sex determination. By cultivating fern prothalli under unfavorable conditions of nutriment, PRANTL (15) was able to confine the production of sexual organs to antheridia. The archegonia demand a prothallus furnished with meristematic tissue, and consequently on a poorly nourished prothallus which has developed no meristem only antheridia can be formed. If prothalli which are producing exclusively antheridia be removed from a culture medium containing no available nitrogen, to a medium in which available nitrogen is present in sufficient amount, meristematic tissue is developed upon which archegonia are formed. KLEBS (12), moreover, has shown that by reducing the amount of light to which they are exposed prothalli may be brought to a prolonged vegetative growth, and thus the formation of both antheridia and archegonia may be suppressed. Professor KLEBS has informed the writer that when the amount of light is increased to a certain extent, antheridia alone are produced from these sterile prothalli,

but that to obtain archegonia, they must be exposed to a still greater illumination. BUCHTIEN (**7**) has shown that in Equisetum external conditions have a similar influence upon the apparent sex of the prothalli.

As yet attempts to influence arbitrarily the sex in unisexual plants have entirely failed. Even though it remain impossible to change the sex in the thalli of Marchantia, it may be found that, by experimenting on the sporophyte where we must assume the sex is unsegregated, one may be in a position to bring about the exclusive production of either male or female spores in a given sporangium. Such a result if accomplished would be analogous to the suppression of one set of sexual organs on the prothalli of ferns.

The behavior of the gametophyte of homothallic ferns and that of the sporophyte of such heterophytic flowering plants as *Melandrium album* (**19**) shows that, abnormally in certain forms and normally in others, only one sex may make its appearance. The conclusion suggested by an assemblage of facts, especially from the animal kingdom, is generally accepted that in so-called unisexual forms one sex is dominant and finds expression in the formation of gametes or spores of the given sex, while the opposite sex exists in a latent condition. However probable such a conclusion may appear for the majority of forms investigated, it must be admitted as at least a possibility that in certain plants or in certain stages a single sex may exist in a pure condition. The fact that besides the occasional production of unisexual germ tubes the zygote of Phycomyces gives rise typically to germ tubes in which the differentiation of sex has not taken place is proof neither for nor against the purity of the male and female thalli, and suggests that the not infrequent occurrence among heterophytic flowering plants of individuals with male and female flowers is as much an indication that both pure and mixed conditions may exist in the sporophyte of these plants as a proof that in heterophytic plants the opposite sex always exists in a latent condition. The germinations of the zygotes of Phycomyces and Marchantia suggest the possibility that the sex may be pure in the gametophyte while mixed in the sporophyte. The observations on unisexual plants, however, have been as yet confined almost entirely to the sporophytic stage, and little is known as to how strict

the differentiation of sex actually is in plants in the gametophytic stage.

Unless the gametes contain both sexes, parthenogenesis in homothallic forms should give rise to unisexual individuals—the male gamete to male and the female gamete to female individuals. So far as the writer is aware, no investigations have been undertaken with a view to confirm this assumption experimentally. Attempts made by the writer to determine the sexual character in the gametes of homothallic mucors by means of their germination before or after their transformation to azygospores have not as yet been successful. In the higher plants, parthenogenesis in the sense of the development of an individual from a sperm or egg cell with the reduced number of chromosomes is, so far as the writer is aware, not definitely known to occur. The sex in the apogamous seeds of Taraxacum for example, however, must contain male characters if the plants produced from them develop stamens, as seems regularly to be the case.

What the essential difference between sex actually is, is as yet beyond conjecture, and the significance of sex in organic development is at present a subject of conflicting discussion. It is to be hoped that a further study, especially of lower forms, where the gametes are more closely connected with the vegetative portions and the zygotes formed by their union more accessible to manipulation, may lead to a better understanding of some of the fundamental problems of sexuality. The present brief article is no place for any detailed discussion of sexuality in the various groups of plants. For a short general presentation of the subject, the reader may refer to the recent work of KÜSTER (**13**) and to the literature therein cited. It seems not out of place, however, to say a few words in regard to the thallic differentiation in the lower cryptogams, where the subject has received little attention.

Unisexual and bisexual forms occur throughout the plant kingdom, and are often to be found in the same genera. This sexual differentiation seems to have no relation to the stage of phylogenetic development. Thus while in higher animals the unisexual condition predominates, in higher plants the monoecious, *i. e.* homophytic, condition is the more common. Again, the majority of the ferns

are homothallic, while the majority of the mucors investigated are heterothallic. Both conditions, therefore, may be expected *a priori* in any group under investigation, whatever may be its phylogenetic rank.

In groups in which sexuality is present, in both fungi and algae, there are many forms for which the sexual spores have been but rarely found or are entirely unknown. The absence of sexual reproduction may be due (1) to constitutional sterility, (2) to conditions of growth unfavorable to the production of sexual organs, or (3) to the fact that the form is heterothallic and thalli of both sexes have not been found together. In the last case the apparent sterility would not be due to a lack but rather to an excess of sexuality which separates the male and female individuals. Even in heterothallic species, neutral races have been found to exist, and the conditions within which sexual reproduction is possible are frequently very limited.

A morphological inyestigation may suffice to show that the male and female organs are borne on the same thallus, and the form in question can then be at once classified as homothallic. A heterothallic condition, on the other hand, can never be recognized by a morphological investigation alone. The appearance of but one set of sexual organs on an individual form studied under the microscope may be due either to dichogamy or to suppression of the other sex brought about by conditions of growth, as well as to a unisexual character of the thalli. Carefully conducted cultures are therefore essential to a determination of the sexual character of forms investigated. A few examples may be briefly given to illustrate the necessity of employing the cultural method in a study of even well-known forms. Many other examples equally as appropriate will suggest themselves to the reader.

In the mosses the leafy shoots arise from an inconspicuous protonema, and if certain shoots bear only antheridia and others only archegonia, a cursory investigation would lead one to consider the forms heterothallic, especially if the antheridial and archegonial "plants" differ in appearance. *Funaria hygrometrica*, for example, is classified as monoecious bv LIMPRICHT (**14**) and CORRENS (**10**), yet CAMPBELL (**8**, p. 187) says "Funaria is strictly dioecious." The

term here is perhaps used in reference to the constant separation of the sexual organs on different shoots without regard to their ultimate connection on the protonema; yet the latter is as an essential part of the plant as the leafy axis, and if the species is in fact homothallic it is not to be called dioecious. Such forms as Funaria offer an interesting field for regeneration experiments to determine if protonemata developed from antheridial and archegonial shoots differ at all in sexual character.

Among the algae, Spirogyra, to mention a simple example, is a familiar genus in which homothallic species are known to occur, and in which heterothallism is strongly to be suspected for certain species from a mere morphological investigation. In *fig. 1*, which is taken from STRASBURGER'S textbook, is represented *Spirogyra longata*.

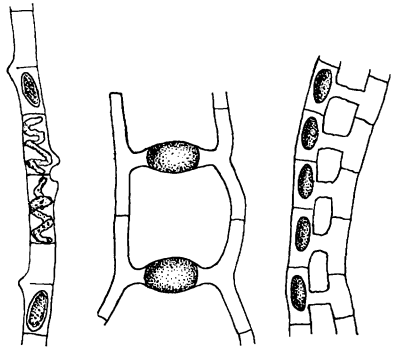

FIG. 1 Spirogyra FIG. 2 Debarya FIG. 3 Spirogyra

It seems in this type to be a matter of indifference whether the two conjugating cells come from the same or from different threads. Obviously here the filaments are bisexual and the species is therefore homothallic. In Debarya, represented in *fig. 2*, the zygospores are formed, as in the heterothallic mucors, midway between the two thalli, between which no differences are apparent. In the most common form of conjugation, however, which is represented in *fig. 3*, one filament seems to be receptive, since it contains all the zygotes formed between two conjugating filaments and has therefore been considered female. Though rather improbable, it is yet imaginable that a filament which acts as female toward one thread might function as male toward another. Theoretically it would not be a difficult task to determine by cultivation the sexual character of any form found producing zygospores.

The Saprolegniaceae form sexually one of the most interesting groups among the fungi. In *Achlya racemosa* the antheridial branches are borne from the stalk of the oogonium, in *A. polyandra*

they arise fom differentiated branches which are only distantly connected with the hyphae which bear the oogonia, and in *Saprolegnia dioica* and *S. anisospora* we have forms which have been described as dioccious. Cultural investigation alone can determine whether these latter forms are in fact heterothallic. It is perhaps significant that in this group forms have been found which have remained sterile under cultivation (*cf.* HORN, II. p. 232). It is not improbable that they may represent unmated strains of heterothallic species.

Of especial interest will be an investigation for the possible occurrence of two sexual races in groups such as the desmids, the flagellates, and the infusoria, where the whole vegetative organism functions directly as the gamete.

Among the cryptogams, with the exception of the mucors and Marchantia, the sexual relations of the offspring from a single zygote in heterothallic forms, the zygotes of which give rise to more than a single individual, have never been investigated. The condition in the bryophytes has been already discussed under Marchantia. In the thallophytes writers see an alternation of generations variously expressed or suggested in the interpolation of carpospores between the fertilized zygote and the young plant. Whether in the heterothallic oedogoniums, to mention but a single example, the four carpozoospores formed at the germination of the oospore are always all of the same sex, like the germ spores in *Mucor Mucedo*, or may be some male and some female, like the germ spores in Phycomyces, can be decided only by an investigation of the individual thalli which they produce. If species in the Saprolegniaceae and Peronosporeaceae are found to be heterothallic, these forms will likewise furnish a fruitful field for investigation.

The discussion in the foregoing pages is based for the most part upon investigations done or already in progress in the Botanical Institute in Halle. The writer wishes to express his grateful appreciation to Professor KLEBS for the facilities of the laboratory and for his unfailing sympathy in the researches undertaken.

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